conditions." What is crucial is not the ALT level but whether to treat the patient if his/her liver disease is not severe.

Future antiviral therapy for hepatitis C

IFN plus ribavirin combination therapy brought about substantial improvement in comparison with the IFN therapy introduced in the 1990s. This combination may lead to high viral elimination primarily because it decreases the incidence of relapse in patients who have become HCV-negative during the therapy. According to an analysis of patient characteristics by the aforementioned Japanese clinical study[33] of PEG-IFNα-2b plus ribavirin combination therapy for genotype 1b patients with high viral load, SVR rates in treatment-naïve patients, relapsers, and nonresponders were 43.1% (59/137 patients) 62.6% (57/91 patients), and 19.2 (5/26 patients), respectively. The fact that relapsers achieved higher SVR rates than treatment-naïve patients suggests that PEG-IFN plus ribavirin combination therapy maximizes the therapeutic effect of IFN and encourages complete viral elimination in IFN-responding patients. On the other hand, the low SVR rate in nonresponders indicates that PEG-IFN plus ribavirin combination therapy is not always useful in patients who do not respond to IFN. To improve SVR rates in such patients, more-effective antiviral agents other than IFN must be developed. Furthermore, as described earlier, PEG-IFN plus ribavirin combination therapy induces a variety of adverse effects. Clearly, safer and better tolerated therapies are needed.

Promising agents for future anti-HCV therapies are classified as HCV-specific inhibitors targeting its protease and polymerase activities, IFN inducers, or less-toxic ribavirin-like agents. A number of drugs are in preclinical or clinical trials.

HCV protease inhibitors

HCV encodes at least four enzymes required for virus replication. They include NS2/3 autoprotease, NS3 helicase, NS3/4A serine protease, and NS5B RNA-dependent RNA polymerase. Intensive work on developing specific inhibitors has focused on the last two.

SCH 503034 is a novel, orally active HCV protease inhibitor that exhibits potent and specific antiviral activity in HCV replicon assays. Recently, a phase 1b clinical trial was conducted for both monotherapy[53] and combination therapy with PEG-IFNα-2b.[54] SCH 503034 exhibited dose-dependent HCV antiviral activity in genotype 1 patients in whom PEG-IFN therapy had previously been unsuccessful. In combination with PEG-IFNα-2b, SCH 503034 had at least an additive effect on HCV suppression. VX-950 is an orally administered highly selective peptidomimetic inhibitor of HCV NS3/4A protease. In a phase 1b clinical trial, VX-950 was well tolerated for 5 to 14 days in both healthy subjects and patients with viral genotype 1, with no serious adverse effects. VX-950 showed a 4.4-log reduction in median HCV RNA at the end of 14 days of therapy.[55]

In addition of its critical role in virus replication, the NS3/4A protease also plays a role in suppressing the cellular antiviral response. Active NS3/4A prevents the phosphorylation and activation of interferon regulatory factor (IRF)-3 and the triggering of downstream IFN-induced antiviral effector genes.[56,57] IRF-3 activity has been shown to be restored by a HCV protease inhibitor. Thus, an effective protease inhibitor may block not only RNA replication but also the ability of HCV to evade innate antiviral responses.

HCV polymerase inhibitors

Valopicitabine (NM283) is a 3′-valyl prodrug of a nucleoside analog that exhibits anti-HCV activity via inhibition of viral RNA polymerase. Valopicitabine is currently in phase 2 clinical development for the treatment of chronic hepatitis C. In a phase 2a trial, valopicitabine demonstrated potent anti-HCV activity when administered in combination with PEG-IFNα-2b, with 4.5-log serum HCV RNA reduction at 6 months and no obvious viral breakthroughs. In a phase 2b clinical trial, the combination therapy was also effective for patients previously unresponsive to PEG-IFN and ribavirin combination therapy.[58]

Since HCV has a higher intrinsic mutation rate than HIV, resistance is expected to be a problem with the use of any type of HCV-specific inhibitor targeting NS3/4A or NS5B proteins. To suppress the risk of a possible escape mutant, combination therapy with PEG-IFN may be better than monotherapy because the former can more efficiently suppress the levels of HCV replication. In the future, a combination of two or three different types of HCV inhibitors may offer a promising approach, similar to HIV cocktail therapy.

Immune modulators

Successful spontaneous clearance of HCV infection is thought to require both innate (e.g., direct antiviral activities by cytokines and natural killer cells) and adaptive (T cell-mediated) immune responses. Chronic HCV infection is characterized by an inadequate immune response that fails to clear the virus.[59] Immune modulators, alone or in combination with direct antiviral agents such as IFN and HCV inhibitors, represent a possible opportunity to improve HCV clearance.

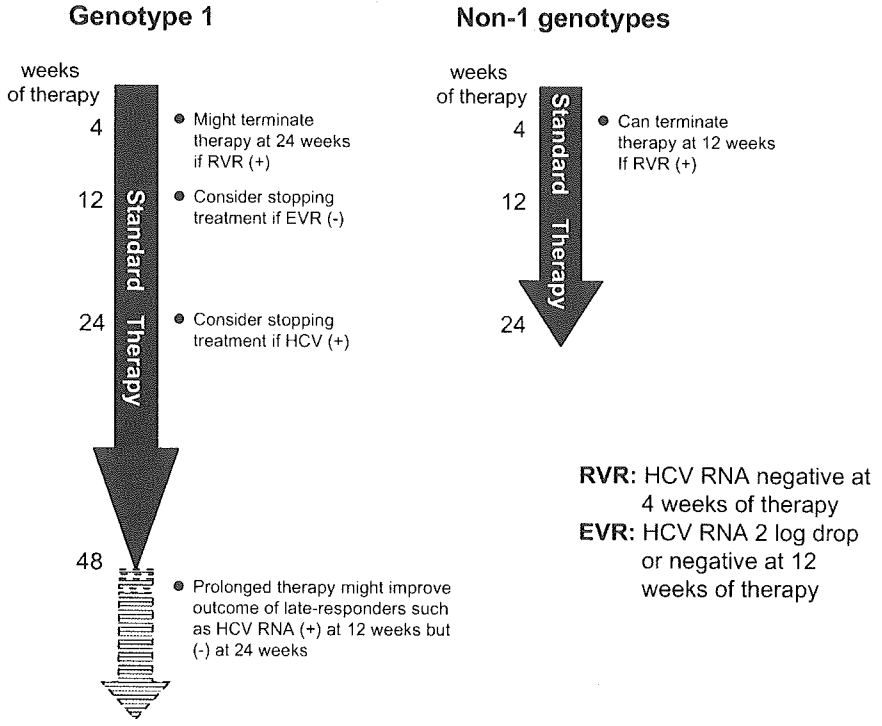

Fig. 3. Various treatment regimens of pegylated IFN and ribavirin combination therapy. *RVR*, rapid virologic response; *EVR*, early virologic response

CPG 10101 is a synthetic agonist of toll-like receptor (TLR) 9. HCV-infected patients receiving CPG 10101 subcutaneously had a more than 1-log reduction in HCV viral load while on therapy.[60] Further development of this agent will continue in conjunction with PEG-IFN and ribavirin.

Isatorbine is a TLR7 agonist. In a proof-of-concept clinical study, intravenous injection of isatorbine once daily for 7 days to patients chronically infected with HCV yielded a significant reduction of serum HCV RNA that correlated with induction of 2′,5′-oligoadenylate synthetase. Recently, the orally available prodrug of isatorbine, ANA975, was developed and studied in healthy phase 1 volunteers and showed promising pharmacokinetics and tolerability.[61]

Ribavirin-like agents

The addition of ribavirin to IFN therapy more than doubled the SVR rate, although its mechanism of action is unknown.[62] Furthermore, higher doses of ribavirin clearly improved response rates in genotype 1 patients.[32,63] However, ribavirin-induced hemolytic anemia is a major obstacle to implementation of a higher dosage regimen and limits its use in patients with comorbidities. To develop a better tolerated combination therapy, ribavirin-like agents lacking a hemolytic effect are needed. Viramidine is a ribavirin prodrug that is metabolized primarily in the liver. In a phase 2 study, fewer patients receiving viramidine developed anemia compared with those given ribavirin, but they also showed lower SVR rates.[64] Phase 3 trials have been undertaken of both PEG-IFNα-2a and PEG-IFNα-2b combined with viramidine in comparison with the combination with ribavirin.

Conclusion: viewpoints other than SVR

IFN treatment of patients with chronic hepatitis C were initially based on observations of its biochemical effects, before the discovery of HCV. Subsequently, evaluation of SVR at 6 months after stopping therapy as a clear end point made it possible to assess therapeutic results in a scientific manner. IFN therapy has been developing over the past decade, with the aim of improving the SVR rate, and higher rates are expected to be achieved with new, more specific antiviral agents.

The question arises as to what the ultimate purpose of hepatitis C treatment is. The answer is that it is the prevention of liver-related death of HCV-infected patients by suppressing progression to decompensated liver disease and liver carcinogenesis (Fig. 4), meaning that hepatitis C is not just an infectious disease, but a potentially serious liver disease. From this point of view, SVR is no more than a surrogate marker—albeit a very strong one—to improve the prognosis of HCV-infected patients. Hepatocellular cancer occurs even in patients

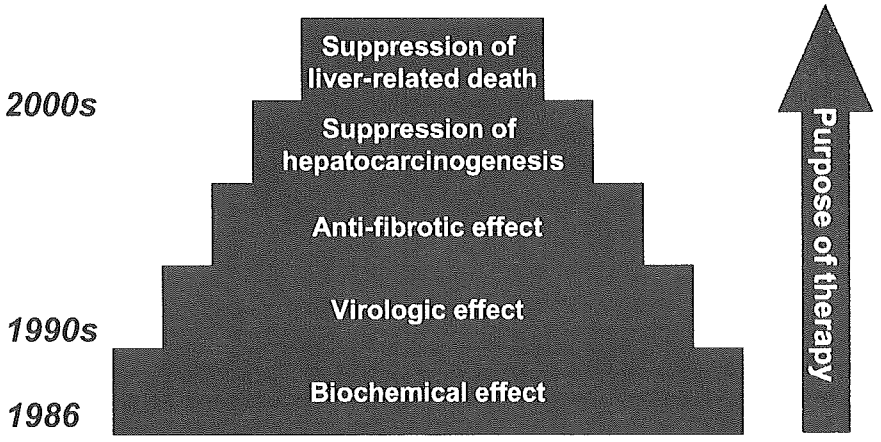

Fig. 4. Effects of IFN-based therapy in relation to the purpose of treatment for chronic hepatitis C. Retrospective analysis of rates of hepatocellular carcinoma and liver-related death after IFN monotherapy have shown a reduction in risk, especially in patients with moderate liver fibrosis[65–69]

who have experienced SVR, although its incidence is substantially lower in those patients than that in untreated patients or nonresponders. Thus, routine hepatocellular cancer screening is essential even after patients have experienced SVR, and early treatment is indispensable if it occurs. On the other hand, the cumulative incidence of hepatocellular carcinoma is clearly suppressed around half in even relapsers at least for 5 years after the termination of therapy compared with that in untreated patients.[65] Therefore, the therapeutic effect of IFN therapy should be evaluated not only on the basis of the SVR rate but also from the more important viewpoint of inhibition of hepatocellular cancer. In this context, repeated IFN therapy, for example every 5 years, for relapsers, and long-term, low-dose IFN therapy for nonresponders should also be considered until a new era dawns of treating hepatitis C with novel anti-HCV agents.

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J Iwate Med Assoc Vol. 57, No. 4 (Oct 2005) pp. 419-426.

Genotypes of hepatitis B virus (HBV) and those clinical characteristics in HBV carrier residents in Iwate, Japan: Results from health-screening program

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岩手県におけるB型肝炎ウイルス（HBV）キャリア住民のHBV遺伝子型とその臨床的特徴

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(Received on March 2, 2005 & Accepted on March 10, 2005)

Abstract

Recent studies indicate that genotypes of hepatitis B virus (HBV) are closely associated with pathogenesis and clinical outcome in HBV related liver diseases. However, the prevalence of these HBV genotypes and clinical features in HBV carriers remain unclear. From 1977 to 2004, a health-screening program in Iwate Prefecture identified 6,711 HBV carriers. A sample of 661 cases (male, 343; female, 318) was extracted using a stratified random sampling method. In addition, further 30 HBV carriers complicated with HCC during long-term follow-up were identified from the registration system for cancer patients of Iwate Prefecture, and they were enrolled in the study. HBV genotypes were determined using an enzyme linked immunosorbent assay kit. Distribution of HBV genotypes showed 19 (2.9%) with genotype A, 297 (44.9%) with genotype B, and 345 (52.2%) with genotype C. In 30 cases with HCC, genotype B and genotype C were 30% and 70%, respectively. HBV genotypes are closely associated with age, positive rate of HB e antigen (HBeAg), and fluctuation of serum alanine aminotransferase (ALT) levels during a follow-up. Cases that developed HCC with genotype B were found to be significantly older compared to genotype C. In conclusion, the prevalence of genotypes B and C were equal in HBV carriers residing in Iwate Prefecture. Differences between HBV genotypes, in particular genotypes B and C, were closely associated with positive rate of HBeAg, fluctuating serum ALT levels, and clinical outcomes of these carriers.

Key words : health-screening, HBV carrier residents, HBV genotype, HB e antigen, hepatocellular carcinoma

I. Introduction

In Asian and African countries, Hepatitis B virus (HBV) infection is still a major cause of acute hepatitis (AH) and various forms of chronic infection, including asymptomatic HBV carrier state, chronic hepatitis (CH),

liver cirrhosis (LC), and hepatocellular carcinoma (HCC), because infection occurs predominantly through perinatal transmission to babies from carrier mothers who are positive for hepatitis B e antigen (HBeAg) in serum or through horizontal spread during infancy. Eight genotypes of HBV from A to H are currently recognized[1, 2] and have distinct geographical distributions[3-7]. In general, while genotypes B and C frequently occur in Asia[4-6], genotypes A and D are common in Western countries[7]. In Japanese patients with chronic HBV infection, the geographic distribution of genotypes B and C also differs among regions of Japan[6]. In particular, genotype B is relatively frequent in the Tohoku region (the northern part of mainland Japan), which includes Iwate Prefecture, compared to other regions except the Okinawa region. Many recent studies have shown that HBV genotypes are closely associated with clinical manifestations and outcome in patients with acute or chronic liver diseases[8-10]. Furthermore, recent reports have also demonstrated that the differences between genotypes B and C, and subtypes of genotype B are considered as causative agents of HCC[5, 10-13]. However, these data were primarily obtained from inpatients and outpatients who visited one hospital.

Approximately one million people are estimated to be HBV carriers in Japan. The majority of these HBV carriers are receiving no medical treatment or follow-up care. A small number of these HBV carriers who are HBeAg positive or have abnormal levels of serum alanine aminotransferase ALT are recognized as a high-risk group for developing CH and LC or HCC[5, 14-16]. However, to ensure precise health management for all HBV carriers, it is important to clarify the differences between clinical characteristics of all HBV carriers with different genotypes.

In the present study, we clarify: 1) the prevalence of HBV genotypes in HBV carriers identified during a health-screening program in Iwate Prefecture; 2) the relationship between HBV genotypes and age; 3) the relationship between HBV genotypes and rate of positive HBeAg and changes of serum transaminase levels during long-term follow-up; and 4) the prevalence of HBV genotypes and the clinical profiles of HBV carriers complicated with hepatocellular carcinoma during a long-term follow-up.

II. Subjects and Methods

1. Subjects

From 1977 to 2004, a total of 381,601 Japanese residents (male: 172,961; female: 208,640) of Iwate Prefecture received a health screen at the institute of Iwate Healthy Science Association. Among these individuals, 6,711 residents (male: 3,576; female: 3,135) were diagnosed as HBV carriers. Using the stratified random sampling method, 661 HBV carriers (male: 343; female: 318) were recruited into samples of approximately equal age and number of individuals for a follow-up every six months or annually by the Iwate Healthy Science Association until 2004. Further 60 HBV carriers complicated with HCC during a follow-up were also recruited from the Registration System for Cancer Patients of Iwate Prefecture. However, only 30 cases with HCC were examined in this study because blood samples could not be obtained from all cases.

Demographic information for HBV carriers

Table 1. Demographics of HBV carriers with or without HCC

	Cases without HCC	Cases with HCC
Total number of HBV carriers	661	30
Sex (male:female)	343:318	21:9
Median age (years, range)	41.9 (16~80)	47.4 (35~67)
Male	41.3 (16~75)	47.0 (35~65)
Female	42.9 (19~80)	48.6 (35~67)
Median follow-up period (years, range)	10 (2~26)	8 (2~15)
Male	9 (2~26)	8 (2~15)
Female	10 (2~26)	9 (4~13)
Serum transaminase levels (mean ± SD)		
AST (IU/L)	24.2 ± 23.0*	54.3 ± 41.2
ALT (IU/L)	29.9 ± 23.6*	38.3 ± 33.3

* $p<0.05$ (compared to cases with HCC)

with or without HCC are shown in Table 1.

The study protocol was approved by the Human Ethics Review Committee of Iwate Medical University, and was permitted by the Committee of Iwate Health Service Association and Iwate Medical Association.

2. Methods

We used the initial serum samples for determination of HBV genotypes which were stored at −20℃ in the institute of Iwate Health Service Association.

HBV genotypes were determined using an enzyme linked immunosorbent assay (ELISA) kit (Institute of Immunology Co., Ltd., Tokyo, Japan) according to the method previously reported by Usuda, et al.[17]. Briefly, 10 μl of a serum sample was placed on a plate fixed with monoclonal antibodies against epitope *b* (located in the pre-S antigen of HBV, and common to all genotypes), epitope m (specific to genotype B), and epitopes *k*, *s* and *u* (associated with several genotypes). A reactive enzyme was then added for color development, and absorbency was measured to determine HBV genotype.

HBsAg was determined using commercial hemagglutination assay kits (MyCell, Institute of Immunology Co. Ltd., Tokyo, Japan). HBeAg and anti-HB e antibody (anti-HBe) were also determined using commercial enzyme immunoassay kits.

Serum levels of ALT, asparate aminotransferase (AST) and γ-glutamyltransferase (γ-GTP) were examined using a routine automatic analyzer.

3. Statistical analysis

Data are expressed as mean±standard deviation (SD) or median (range). Comparisons between the groups were performed by Chi-square test of Fisher's exact test. Probabilities of less than 0.05 were considered statistically significant.

III. Results

1. Distribution of HBV genotypes and their relationship with age

Of 680 cases, 19 (2.9%) were genotype A, 297 (44.9%) were genotype B, and 345 (52.2%) were genotype C. The 30 cases with HCC showed 9 (30%) cases of genotype B and 21 (70%) cases of genotype C. Genotype A was not detected in HCC cases.

Table 2. The relationship between positive rate of HBeAg in each genotype and age at first examination

	Genotype A	Genotype B	Genotype C
Total numbers	19	297	345
Sex (male:female)	13:6	157:140	173:172
Numbers of HBeAg positive (%) #	0 (0)	11 (3.7)	115 (33.3) $^{++}$, **
Sex (male:female)	0:0	8:3	66:49
>29 years	0 (0)	7 (24.1)	29 (43.9)
30 ~39	0 (0)	2 (3.3)	37 (33.0)
40 ~49	0 (0)	0 (0)	28 (28.9)
50 ~59	0 (0)	1 (1.2)	13 (26.5)
60 ~69	0 (0)	1 (2.9)	7 (36.8)
70<	0 (0)	0 (0)	1 (50.0)
Liver function tests			
AST (IU/L)	22.4 ± 11.1	25.8 ± 12.7	33.8 ± 29.9^{+}, *
ALT (IU/L)	29.3 ± 22.1	27.7 ± 17.5	40.8 ± 50.0
g-GTP (IU/L)	29.4 ± 22.3	28.9 ± 40.9	36.2 ± 47.2

$^{+}$, * $p<0.01$ (compared to genotypes A and B, respectively)
$^{++}$, ** $p<0.001$ (compared to genotypes A and B, respectively)
Percentage is the rate of HBeAg positive cases among the total number of HBV carriers in each age group

The relationship between each HBV genotype and age is shown in Figure 1. When the age of HBV carriers was compared based on age in 2003, genotype A was found only in the 20～30-year-old carriers. The rate of genotype B gradually increased as age increased and was the highest in carriers over 70 years old. The rate of genotype C was higher in 40 to 60 years old, but lower in carriers over 70 years old.

2. Positive rate of HBeAg in each HBV genotype

The positive rate of HBeAg for each genotype of HBV was 0% in genotype A, 3.7% in genotype B and 33.3% in genotype C. The positive rate of HBeAg in genotype C was significantly higher ($p<0.001$) than that in genotypes A and B. The relationship between

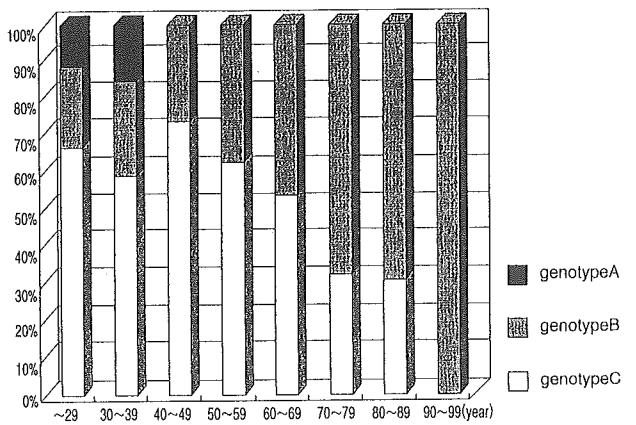

Fig. 1. The relationship between each HBV genotype and age

Table 3. Profiles of HCC cases with genotype B or C

	Genotype B (9)	Genotype C (21)
Age developed to HCC (years)	64 ± 13*	52.7 ± 9
Numbers of HBeAg positive (%)	0 (0%)	6 (27.3%)
Serum ALT levels during follow-up	39.4 ± 22.8	65.5 ± 52.8

* $p<0.05$ (compared to genotype C)

the positive rate of HBeAg in genotypes B and C and age is shown in Table 2. In 20- to 30-year-old genotype B carriers, 11 carriers exhibited a relatively higher positive rate of HBeAg. On the other hand, in genotype C, the percentages of HBeAg positive cases were higher than genotype B at all ages, in particular, in the carriers in their 20s.

3. Changes in the HBeAg/anti-HBe system in genotype B and C carriers during follow-up

Six (54.5%) of 11 genotype B carriers who were positive for HBeAg at the first examination seroconverted to anti-HBe during follow-up, while 5 (45.5%) carriers remained HBeAg positive during follow-up. Of the 115 genotype C carriers who were positive for HBeAg at the first examination, 24 (20.9%) carriers seroconverted to anti-HBe and 12 (10.4%) carriers were positive for alternately between HBeAg and anti-HBe, while 79 (68.7%) carriers remained HBeAg positive during follow-up. Duration until the seroconversion from HBeAg to anti-HBe was 4.5±2.3 years (range; 2 ~ 8 years) in genotype B, and 6.0±4.2 years (1 ~15 years) in genotype C.

4. Relationship between HBV genotypes and serum ALT levels during follow-up

Serum ALT levels during a long-term follow-up were compared among the three HBV genotypes. Serum ALT levels of each HBV genotype were 29.3± 22.1 IU/L in genotype A, 27.7±17.5 IU/L in genotype B, 40.8±50.0 IU/L in genotype C. The levels of serum ALT in the genotype C were significantly higher ($p<0.05$) than those of genotype B, but no significant differences were observed between genotypes A and B.

5. Analysis of HBV carriers complicated with HCC

Comparison between HCC cases with genotype B or C is shown in Table 3. Cases with genotype B were significantly older compared to the cases with genotype C. All cases with genotype B exhibited a negative rate of HBeAg at the start of follow-up, while HBeAg was positive in 27.3% of cases with genotype C. Serum ALT levels during follow-up were significantly higher in genotype C than in genotype B.

IV. Discussion

In Iwate Prefecture, the immunoprophylaxis of perinatal transmission of HBV was started in 1981 and covered more than 60% of all babies by 1986 when it became a mandatory national program[18]. Briefly, 20.7% of HBV carrier mothers were positive for HBsAg and their babies received immunoprophylaxis. As a result of this program, the prevalence of HBsAg decreased from 0.75% in children born between 1978 and 1980 to 0.23% in those born between 1981 and 1985, and further to 0.04% in those born between 1986 and 1990. Therefore, it is believed that the prevention of

perinatal HBV transmission influences not only mother-to-baby transmission but also horizontal transmission from HBV carrier children who might be infected during infancy. However, there are still many HBV carriers who have not received immunoprophylaxis under the national program for prevention of HBV infection.

Among the HBV carriers residing in Japan, a small number of HBeAg positive carriers or carriers with abnormal levels of serum ALT are recognized as a high-risk group for developing CH and LC or HCC[5, 14-16]. However, clinical features and outcome in the majority of HBV carriers with different HBV subtypes remain unclarified.

In this large-scale survey three genotypes were found among HBV carriers residing in Iwate Prefecture who were identified during a health-screening program between 1977 and 2004. HBV genotypes B and C were the most common, followed by genotype A. In a previously published report by Orito and colleagues[6], concerning the geographic distribution of HBV genotypes among patients with chronic liver disease in Japan, the prevalence of genotype B in the Tohoku area including Iwate Prefecture was reported to be higher (22.9%) than that of the other mainland areas. However, the rate of HBV genotype B in this study was higher than expected (44.5%), even if a high rate of HBV genotype B was considered to be endemic in the Tohoku area. A possible reason for this is that our subjects lived without any medical management.

Generally, in HBV carrier residents with genotype B, seroconversion from HBeAg to anti-HBe occurs at a young age (10-20 years after birth), resulting in stabilization of liver function. Therefore, the majority of HBV genotype B carriers live as asymptomatic carriers who do not require medical management or follow-up[16]. Actually, the positive rate of HBeAg in carrier residents with genotype B was extremely low compared to carrier residents with genotype C at the start of follow-up. On the other hand, it has been found that seroconversion from HBeAg to anti-HBe at a young age is not frequent in genotype C carriers, and in most patients abnormal serum ALT levels remain[5, 6, 16]. Therefore, the HBV carriers with genotype C have many opportunities to visit the hospital and receive the medical management, resulting in high prevalence of genotype C. In the present study, the prevalence in every age group of genotype C carriers were significantly lower than rates of anti-HBe of genotype B carriers, while the serum AST and ALT levels were higher in genotype C. Ishikawa, et al.[16] previously showed that the seroconversion from HBeAg to anti-HBe was less likely to occur in genotype C carriers, especially in carriers in their 40s. These subjects were also more likely to develop chronic liver disease, because their serum transaminase levels fluctuated and their HBV-DNA levels were high.

Interestingly, in the present study we found that the prevalence rate of genotype A was relatively high among young people from 20 to 30 years old. Genotype A is the predominant genotype in Europe and the United States[2]. A previous report concerning the geographic distribution of HBV genotypes in Japan showed a low prevalence rate (1.7%) of HBV genotype A[6]. A recent report has suggested that acute hepatitis patients infected with HBV genotype A often transfer to a persistent

HBV carrier state[19]. Also, in Europe, most HBV infections are genotypes A and D, and significantly more genotype A carriers developed chronic liver disease when compared with genotype D carriers[20]. The reason for the increased prevalence rate of genotype A in Iwate Prefecture among the young generation is unclear, and it is therefore necessary to follow these carriers over the long term.

HBV is one of the major causative agents of HCC in Japan. In particular, HBV genotypes B and C are frequently seen in patients with HCC. Previous reports in Japan showed that the mean age is higher in HCC patients with genotype B than in those with genotype C, although results in Taiwan and another Asian countries are controversial [5, 11-13]. In general, genotype B is less prevalent than genotype C among patients with liver cirrhosis, because HBV genotype B is associated with earlier seroconversion from HBeAg to the corresponding anti-HBe and with lower histological activity scores. In the present study, we also demonstrated that HBV carriers with genotype B and HCC were significantly older than cases with genotype C. In addition, genotype B carriers showed lower serum ALT levels during follow-up than genotype C carriers. Therefore, these results suggest that genotype C carriers might have a tendency for persistent fluctuation of abnormal serum ALT levels over the long-term, accelerating the development of HCC.

Recently, lamivudine, an oral cytosine nucleoside analogue, which potently inhibits HBV replication by interfering with HBV reverse transcriptase activity, has been used clinically for the treatment of chronic HBV infection[21-23]. This therapy for chronic HBV infection induced a marked decrease in HBV-DNA and ALT levels, resulting in histological improvement, although lamivudine-resistant HBV strains have appeared in long-term lamivudine therapy[24, 25]. Therefore, this therapy is expected to change the natural course of HBV carriers with persistent abnormal liver function.

In conclusion, the prevalence of genotypes B and C were equal in HBV carriers residing in Iwate Prefecture. Differences between HBV genotypes, in particular genotypes B and C, were closely associated with positive rate of HBeAg, fluctuating serum ALT levels, and clinical outcomes of these carriers.

The authors would like to thank Prof. S. Horiuchi (Department of Biochemistry, Iwate Medical University, School of Medicine) for her helpful advice and comments.

内容自抄

B型肝炎ウイルス（HBV）には８つの遺伝子型（A～H）が存在しているが，HBVキャリア住民の遺伝子型頻度や自然歴は十分に検討されていない．そこで，検診受診者を対象にHBVキャリアの遺伝子型とその臨床的特徴を検討した．岩手県予防医学協会でHBVキャリアと診断された661例と岩手県癌登録事業より肝細胞癌で死亡が確認された30例を対象とした．遺伝子型の測定はELISA法を用いた．岩手県のHBVキャリア住民の遺伝子型の頻度は，各遺伝子型と年齢との関係をみると，Aは20～30歳代でのみ認められ，Bは70歳以上で高く，Cは40～60歳代では半数以上を占めた．各遺伝子型のHBe抗原陽性率の頻度は，CがA，Bより高率であった．経過観察期間中の血清ALT値は，Cが，A，Bに比較して有意に高値であった．肝癌例ではBでの発癌年齢はCに比較して有意に高齢であり，経過観察期間中の血清ALT値も低値であった．

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Original Paper

Intervirology

Intervirology 2005;48:239–245
DOI: 10.1159/000084601

Received: July 14, 2004
Accepted after revision: September 6, 2004

Differences of Hepatocellular Carcinoma Patients with Hepatitis B Virus Genotypes of Ba, Bj or C in Japan

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Key Words
Hepatocellular carcinoma, epidemiology · Subtypes Ba/Bj, hepatitis B · Hepatitis B virus, genotypes B/C

Abstract
Hepatitis B virus (HBV) genotypes B (HBV/B) and C (HBV/C) are prevalent in Asia. Recently HBV/B has been classified into two subtypes, HBV/Ba which is ubiquitously found in Asia, and HBV/Bj which is specific in Japan. In addition, the frequency of positive HBeAg has been reported to be higher in patients with HBV/Ba than those with HBV/Bj. However, little is known about the differences between patients with various genotypes who developed hepatocellular carcinoma (HCC). In 296 serum samples of HCC patients collected from all over Japan, HBV genotypes were determined with the restriction fragment length polymorphism. HBV/A was detected in 1.0%, HBV/Ba in 4.4%, HBV/Bj in 7.4%, and HBV/C in 86.5%. In the Tohoku district and Okinawa, HBV/Ba, HBV/Bj and HBV/C were found in 6.7, 40.0 and 48.9%, compared to 4.0, 1.6 and 93.2% in the other districts in Japan. HBV/Bj patients were more frequently found in the group older than 65 years while HBV/Ba patients were found in all age groups. The frequency of positive HBeAg in HBV/Bj patients was significantly low compared to that in the other patients. More than 60% of the patients with HCC had cirrhosis as the underlying liver diseases. However, in HBV/Ba patients aged 50 years or younger, 80% of them had chronic hepatitis, while 87.5% of those aged older than 50 years had cirrhosis. These data suggest that great differences exist among patients with HCC infected with different genotypes.

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Introduction

In Japan, in more than two thirds of the patients with hepatocellular carcinoma (HCC) the disease is associated with hepatitis C virus (HCV). However, hepatitis B virus (HBV) is the major causative agent of HCC in Asian countries. All strains of HBV isolated from various countries can be classified into 8 HBV genotypes, HBV genotype A (HBV/A) to HBV/H, according to their phylogenetic relationships [1–3]. It has been reported that the clinical and virologic manifestations of patients with chronic HBV infection show significant differences among the different HBV genotypes [4–6]. In addition, specific distributions of HBV genotypes have been demonstrated among areas and countries [4, 7]. In south-east Asian countries, such as Japan, Taiwan, or China, HBV/B and HBV/C are prevalent [5, 7, 8].

In Japanese patients with HCC, the patients with HBV/B are rare and their mean age is high [7, 9]. However, in Taiwanese patients with HCC, a high proportion of younger patients have HBV/B. Until now, it is still unclear why younger Taiwanese patients with HBV/B develop HCC while Japanese patients with HBV/B rarely develop HCC, only in older age.

Recently, we demonstrated that HBV/B strains should be divided into two subtypes, HBV/Ba and HBV/Bj, according to their genetic relationship, and that HBV/Ba is found ubiquitously in Asian countries while HBV/Bj is found only in Japan [10, 11]. It was reported that HBeAg was found more frequently in patients with chronic infection with HBV/Ba than in those with chronic infection with HBV/Bj (32 vs. 9%) [12]. However, it is still unknown whether etiological and virologic differences are found between the HCC patients with HBV/Ba and HBV/Bj. Thus, in the patients with HCC, the difference between the subtypes of HBV/Ba and HBV/Bj might explain the etiological or clinical differences between Japan and Asia where HBV/Bj and HBV/Ba are endemic, respectively.

So, the aim of this study was to investigate the differences in the etiological, virologic and clinical characteristics among Japanese HCC patients with different HBV genotypes, such as HBV/Ba, HBV/Bj or HBV/C.

Patients and Methods

Patients with HCC

Two hundred and ninety-six patients with HCC were consecutively collected from 19 hospitals throughout Japan during January 2001 to December 2002. All the patients were chronically positive for HBsAg, and negative for anti-HDV, anti-HCV and anti-HIV. The diagnosis of HCC was reached clinically with ultrasound, computerized tomography, magnetic resonance imaging, angiography, tumor markers and biopsy if possible. The diagnoses of chronic hepatitis (CH) and liver cirrhosis (LC) were principally done by liver biopsy. However, a proportion of patients with ascites, jaundice or severe thrombocytopenia were diagnosed by ultrasound, computerized tomography and liver function tests. The serum samples and clinical data were collected from these patients with written informed consent. This study was conducted according to the ethical guidelines in our hospitals.

Virologic Assays

In all serum samples, HBsAg (CLIA, Fujirebio, Japan, detection limit 0.13 ng/ml), HBeAg (CLIA, Fujirebio, Japan) and anti-HBe (CLIA) were tested. Serum HBV DNA was detected by nested polymerase chain reaction (PCR) with the primers derived from the S gene. The patients were not enrolled in this study if the serum HBV DNA was not detected by PCR. The HBV genotype was determined by restriction fragment length polymorphism as described previously [13]. In brief, the S gene of HBV DNA was amplified by nested PCR. Then the products were sequentially digested by the restriction enzyme, *Alw*I, *Ear*I, *Hph*I, *Nci*I and *Nla*IV, respectively. The HBV genotype was determined by the size of the digested PCR product which was electrophoresed on agarose gel. When the test results were inconclusive, the sequences of the S region were determined directly, then the genotype was decided by phylogenetic analysis [13, 14]. When patients were found to have HBV/B, the subtypes Ba and Bj were determined by restriction fragment length polymorphism [11]. In brief, at nucleotide position 1838 in the pre-core region, only A was found in patients with HBV/Ba while only G was found in those with HBV/Bj. The restriction enzyme detection system was established targeting the discrimination of this difference in nucleotides with the restriction enzyme, *Spe*I and *Mse*I after the pre-core region was amplified by PCR.

Statistical Analysis

The data were statistically analyzed by Student's t test, nonparametric Mann-Whitney test, and χ^2 test where appropriate. A p value of <0.05 was regarded as statistically significant.

Results

HBV Genotypes and Clinical Findings

Of the 296 patients, 223 were male and 73 were female. The mean age was 55.1 ± 10.8 (range 26–81) years. The clinical findings are shown in table 1. Thirty-five percent of the patients were positive for HBeAg. Regarding the HBV genotypes, 3 patients (1.0%) were HBV/A, 13 (4.4%) HBV/Ba, 22 (7.4%) HBV/Bj, 256 (86.5%) HBV/C, and 2 (0.7%) of mixed genotype (HBV/B and C). The clinical findings by HBV genotype are shown in table 2. There were no significant differences in the mean levels of total bilirubin, AST and ALT among patients with different HBV genotypes. However, the mean ALP level and γ-

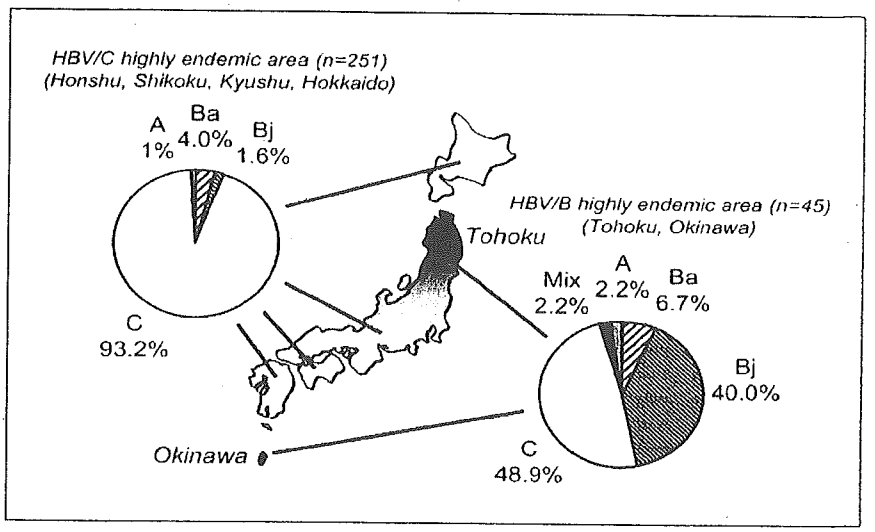

Fig. 1. The geographic distribution of HBV genotypes in Japan. In the Tohoku district, the northern area of mainland Japan, and Okinawa, the most southern islands, 48.9% of HCC patients were HBV/C, 6.7% were HBV/Ba, and 40.0% were HBV/Bj. In contrast, in other parts of Japan, Hokkaido, Honshu, Shikoku and Kyushu, 93.2% were HBV/C, 4.0% were HBV/Ba and 1.6% were HBV/Bj.

Table 1. Characteristics of 296 HBsAg-positive Japanese patients with HCC collected from all over Japan

Male:female	223:73
Age, years	55.1 ± 10.8[a]
Total bilirubin, mg/dl	1.5 ± 1.9
AST, IU/l	78.5 ± 103.9
ALT, IU/l	63.0 ± 69.8
ALP, IU/l	321.1 ± 225.4
γ-GTP, IU/l	108.4 ± 174.4
HBeAg, % positive	35.0
Anti-HBe, % positive	64.8
HBV genotype	
HBV/A	3 (1.0%)
HBV/Ba	13 (4.4%)
HBV/Bj	22 (7.4%)
HBV/C	256 (86.5%)
Mix	2 (0.7%)

[a] Mean ± SD.

Table 2. Clinical findings of the HCC patients with HBV genotypes of Ba, Bj or C

	HBV genotype		
	Ba	Bj	C
Age, years	55.4 ± 12.9	66.6 ± 10.6	54.0 ± 10.7
		p < 0.01	p < 0.01
Total bilirubin, mg/dl	1.0 ± 0.4	1.2 ± 0.7	1.5 ± 2.0
AST, IU/l	173.9 ± 352.6	51.6 ± 42.1	82.6 ± 113.4
ALT, IU/l	102.4 ± 162.9	33.9 ± 16.8	66.5 ± 74.9
ALP, IU/l	147.7 ± 126.6	209.8 ± 95.4	343.9 ± 238.0
		p < 0.05	
γ-GTP, IU/l	78.6 ± 55.9	63.1 ± 45.9	110.5 ± 186.7
			p < 0.05

GTP level of the HBV/C patients was significantly higher than those with HBV/Ba and HBV/Bj, respectively ($p < 0.05$).

Geographic Distribution of HBV Genotypes

The geographic distribution of HBV genotypes was area-specific in Japan (fig. 1). This specific distribution of HCC patients was in accord with that of all the patients including asymptomatic carriers, CH and LC patients, as described previously [7]. Namely, in the Tohoku district, the northern area of the Japanese mainland, and Okinawa, the most southern islands, 22 (48.9%) of HCC patients were HBV/C, 3 (6.7%) were HBV/Ba, and 18 (40.0%) were HBV/Bj. In contrast, in other areas of Japan, Hokkaido, Honshu, Shikoku and Kyushu, 234 (93.2%) were HBV/C, 10 (4.0%) were HBV/Ba, and 4 (1.6%) were HBV/Bj ($p < 0.01$).

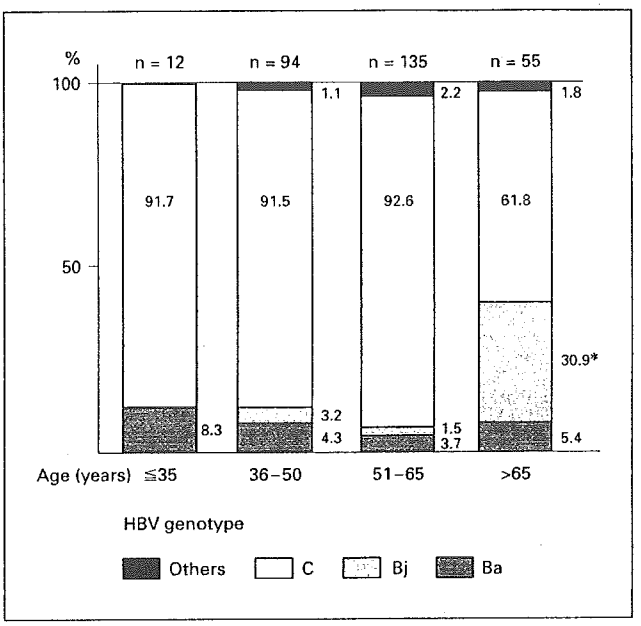

Fig. 2. The distribution of HBV genotypes in each age group. In groups aged 35 years or younger, 36–50 years, and 51–65 years, more than 90% of HCC patients had HBV/C. On the other hand, in the group aged older than 65 years, only 61.8% of patients had HBV/C while 30.9% had HBV/Bj (* $p < 0.01$, group aged older than 65 years vs. other age groups). More patients with HBV/Ba were in the younger aged group, although the number of patients with HBV/Ba was small in all the groups.

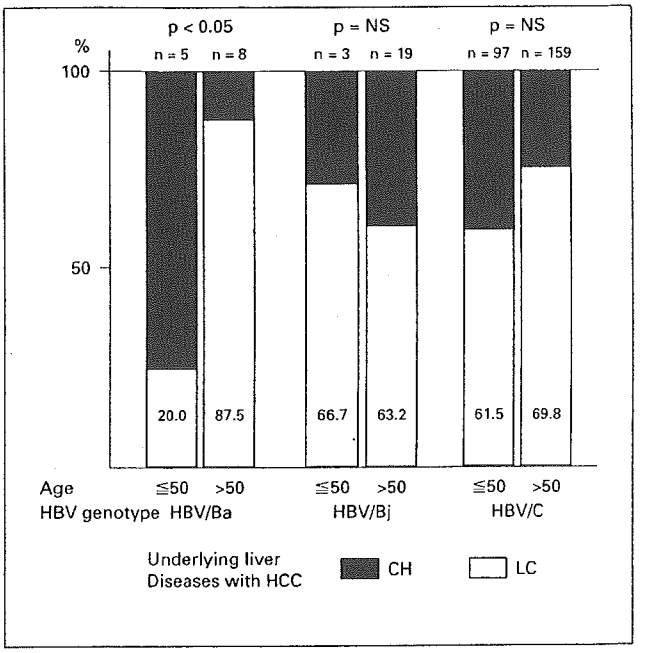

Fig. 4. The underlying liver diseases, chronic hepatitis (CH) or liver cirrhosis (LC), in HCC patients. In patients with HBV/Ba, only 25.0% of the group aged 50 years or younger had LC, while 85.7% of the group aged older than 50 years had LC ($p < 0.01$). However, in patients with HBV/Bj or HBV/C, the ratios of the underlying liver diseases were approximately identical even when compared by age.

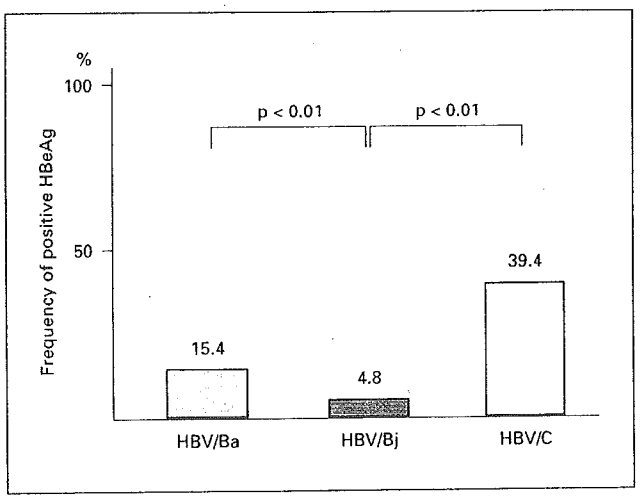

Fig. 3. The frequency of patients with positive HBcAg in each HBV genotype. The frequency of positive HBcAg was 4.8% in patients with HBV/Bj, compared with 39.4% in those with HBV/C (Bj vs. C, $p < 0.01$), and 15.4% in those with HBV/Ba (Bj vs. Ba, $p < 0.01$).

Mean Age and Frequency of Positive HBeAg among Patients with Each Genotype

The mean age of HBV/Bj patients (66.6 ± 10.6 years) was significantly higher than those with HBV/Ba (55.4 ± 12.9 years, $p < 0.01$) and HBV/C (54.0 ± 10.7 years, $p < 0.01$; table 2). The distribution of HBV genotypes in each age group is shown in figure 2. In groups aged 35 years or younger, 36–50 years, and 51–65 years, more than 90% of HCC patients had HBV/C. On the other hand, in the group aged older than 65 years, only 61.8% of the patients had HBV/C while 30.9% had HBV/Bj ($p < 0.01$, group aged older than 65 years vs. other age groups). HBV/Ba tended to be found in the younger age group although the number of patients with HBV/Ba was small in all groups.

The frequency of positive HBeAg was 4.8% in patients with HBV/Bj, compared with 39.4% in those with HBV/C (Bj vs. C, $p < 0.01$), and 15.4% in those with HBV/Ba (Bj vs. Ba, $p < 0.01$; fig. 3).

Underlying Liver Diseases

All HCC patients had underlying chronic liver diseases, such as CH or LC. We compared the underlying liver diseases among those aged 50 years or younger and those aged older than 50 years by HBV genotype (fig. 4). In 13 patients with HBV/Ba, only 1 (20.0%) of the 5 patients aged 50 years or younger had LC, while 7 (87.5%) of the 8 patients aged older than 50 years had LC ($p < 0.05$). However, in patients with HBV/Bj or HBV/C, the ratios of underlying liver diseases were approximately identical even when compared by age.

Discussion

The clinical and virologic features of patients with chronic HBV infection are specific according to their HBV genotypes [4, 15]. However, to date, there has been no report on the relationship between the HBV genotypes of Ba, Bj and C, and the clinical characteristics of HCC patients. We therefore analyzed the relationship between the clinical characteristics of Japanese HCC patients identified throughout Japan, and their HBV genotypes, including the HBV subtypes of Ba and Bj. In this study, we demonstrated that HBV/Ba (4.4%), HBV/Bj (7.4%) and HBV/C (86.5%) were found in Japanese HCC patients, and that there were distinct clinical differences among the three HBV genotypes, in geographic distribution, age distribution, and the frequency of positive HBeAg.

Of the Japanese patients with chronic HBV infection, including asymptomatic carriers, CH, LC and HCC, 1.7% were HBV/A, 12.2% HBV/B, 84.7% HBV/C, 0.4% HBV/D, and the others 1.0%, as reported previously [7]. In this study, we collected 296 serum samples from patients with HCC throughout Japan. In addition, we recently developed a new method for detecting HBV/Ba and HBV/Bj with restriction fragment length polymorphism [11]. Thus, we showed that 1.0% was HBV/A, 4.4% HBV/Ba, 7.4% HBV/Bj, 86.5% HBV/C, and mixed genotype 0.7% in Japanese HCC patients. This prevalence in HCC patients is almost identical to that in all patients with chronic HBV infection [7]. In addition, the geographic distribution of HBV/B and HBV/C in HCC patients is also identical to that in all patients. However, when we analyzed the HBV subtypes of HBV/Ba and HBV/Bj in patients with HBV/B, a high proportion of patients with HBV/Bj is found in the highly endemic HBV/B area, the Tohoku district and Okinawa, while the prevalence of HBV/Ba is approximately identical between the highly endemic HBV/C area, the other areas of Japan, and the highly endemic HBV/B area. Thus, HBV/Bj is specifically distributed in the Tohoku district and Okinawa.

As reported previously, HBV/Ba is ubiquitous in all Asian countries including Japan, although HBV/Bj is specific to Japan and is not found in other countries [11]. In Okinawa, it is reported that a high proportion of patients with chronic HBV infection have HBV/B and a good prognosis compared with patients with HBV/C [16, 17]. In contrast, in Taiwan, close to Japan, a higher proportion of patients aged 50 years or younger with HBV/B have HCC and CH [15]. The underlying liver diseases in those who developed HCC were compared among each HBV genotype group. In the HBV/Ba group, up to 75% of the patients aged 50 years or younger had CH as the underlying liver disease, compared with patients aged over 50 years. On the other hand, in the group with HBV/Bj or HBV/C, more than 60% of the patients had LC regardless of their age. The mean age of the patients with HBV/Ba in Japan is more than 10 years younger than those with HBV/Bj. So, more younger patients with HBV/Ba tend to have CH than the other patients. However, the molecular mechanism is unclear why patients with HBV/Ba develop HCC at a younger age and often have CH.

It is unclear why Japanese patients with HBV/B have a good prognosis while Taiwanese patients with HBV/B often have more advanced liver diseases, such as HCC. The frequency of patients positive for HBeAg in the HBV/Ba and HBV/C groups was higher than in the HBV/Bj group. So, the viral activity of HBV may be higher in patients with HBV/Ba or HBV/C than those with HBV/Bj. Thus, these differences in subtypes of HBV/Ba and Bj could be one of the reasons why the discrepancy in prognosis exists between Japanese and Taiwanese patients with HCC.

The differences in DNA sequences between HBV/Ba and HBV/Bj can be characterized in the core gene [10]. It has been reported that HBV/Ba, not HBV/Bj, recombines with HBV/C in the core gene. The product of the core gene is reported to be a cytotoxic T-cell epitope [18], suggesting that patients with HBV/Ba and HBV/C may be exposed to severe immune responses for destroying hepatocytes compared with those with HBV/Bj. In addition, patients with HBV/Ba more often have core promoter mutations at nucleotide 1762/1764 than those with HBV/Bj [11], which is associated with more advanced liver diseases [6, 19]. Taken together, these facts may indicate a poor prognosis in patients with HBV/Ba compared to those with HBV/Bj.

In the patients with HBV/C, the mean ALP and γ-GTP levels were higher than those with the other genotypes. In this study, there may exist some bias of regarding the tumor size of HCC between patients with HBV/C and the other patients. It is considered that more patients with a rather large size of HCC were found in the patients with HBV/C, resulting in elevation in ALT and γ-GTP levels.

To investigate the hepatocarcinogenesis and risk factors of HCC, it is important to study the differences in host, environmental and viral factors. The various genetic alterations, such as mutations of cancer-associated genes or loss of some chromosomes, are found in the HCC cells [20]. However, the genetic polymorphism varies among populations [21]. The differences in host genomes are still unknown between Japanese and other Asian populations. The association of environmental factors, such as air, water and food contaminated with some chemical agents, and HCC is still unclear, although aflatoxin affects the mutation of p53 in HCC [22]. However, with respect to the viral factors, a survey of the distribution of HBV genotypes or subtypes will be important clues for solving these problems.

Acknowledgements

The authors thank the members of the Japan HBV Genotype Research Group for supporting this study: Dr. Namiki Izumi, Department of Gastroenterology, Musashino Red Cross Hospital, Musashino; Dr. Hiroshi Yotsuyanagi, Department of Gastroenterology, St. Marianna University, Kawasaki; Dr. Hiroshi Yatsuhashi, Department of Clinical Research, National Nagasaki Medical Center, Nagasaki; Dr. Shuhei Hige, Department of Internal Medicine, Hokkaido University Graduate School of Medicine, Sapporo; Dr. Norio Horiike, Third Department of Internal Medicine, Ehime University, Ehime; Dr. Tomoyuki Kuramitsu, Department of Gastroenterology, Akita City General Hospital, Akita;Dr. Kunio Nakane, First Department of Internal Medicine, Akita University, Akita; Dr. Kazuyuki Suzuki, First Department of Internal Medicine, Iwate Medical University, Morioka, and Dr. Keisuke Hino, Department of Gastroenterology and Hepatology, Yamaguchi University Graduate School of Medicine, Yamaguchi, Japan. Finally, this study was supported by grants from the Japanese Ministry of Education, Science, Sports and Culture.

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